X-RAY STRUCTURE ANALYSIS AND NMR STUDY OF SESQUITERPENE LACTONE HIRSUTOLIDE*

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Hirsutolide, the C(8)-lactonized sesquiterpene of the heliangolide type has been studied by X-ray and NMR methods. In crystal the ten-membered ring conformation closely resembles the conformation observed in C(6)-lactonized heliangolides. This conformation, which can be described as a boat-chair is contrasted with the chair-chair conformation found in the crystals of other C(8)-lactonized heliangolide, scorpioidine. Broad signals in ^{1}H and ^{13}C NMR spectra indicated the conformation equilibrium in solution which was confirmed by observation of signals of two conformers (ca 3 : 1) in low temperature ^{1}H NMR spectra. The major conformer is the one present in the crystal structure of hirsutolide and the minor conformer corresponds very likely to the one observed in the crystal structure of scorpioidine.

Sesquiterpene lactone hirsutolide (*IV*) was isolated already twenty years ago from the species *Venidium hirsutum* BEROL. (*Asteraceae* family, *Arctotae* tribe) together with sesquiterpene lactone venidiolide[1]. In our previous work[2] we established that venidiolide is a mixture of two lactones – venidiolide A (*II*) and venidiolide B (*III*) – whose structure we have determined. The structure of hirsutolide (*I*) had been also derived[2] except of the stereochemistry of its epoxy group in position 1,10. Therefore we considered it necessary to complete the structure of hirsutolide by X-ray analysis. In this paper we describe the results of X-ray crystal analysis and new NMR measurement of title compound and we prove that the structure of hirsutolide corresponds to formula *IV*.

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RESULTS AND DISCUSSION

X-Ray Structure Analysis of Hirsutolide

Figure 1 shows a perspective view of the molecule[3]. Table I lists non-H atom coordinates and equivalent isotropic temperature factors. Hirsutolide (*IV*) is a heliangolide type sesquiterpene lactone, as indicated by the *trans*, *cis* orientation of the C-chain substituents at the C(1)–C(10) epoxide equivalent of the double bond, and the C(4)–C(5) double bond, respectively. The epoxide ring at C(1)–C(10) is *trans*, as well as the γ-lactone ring junction at C(7)–C(8).

The ten-membered ring conformation may be described as a boat-chair* and corresponds to the conformation which has been reported as the global minimum energy form of cyclodeca-1,5-diene[4]. The methyl group at C(10) and the methyl ester group at C(4) are *anti*, on the β- and α-face of the molecule, respectively. The spatial arange-

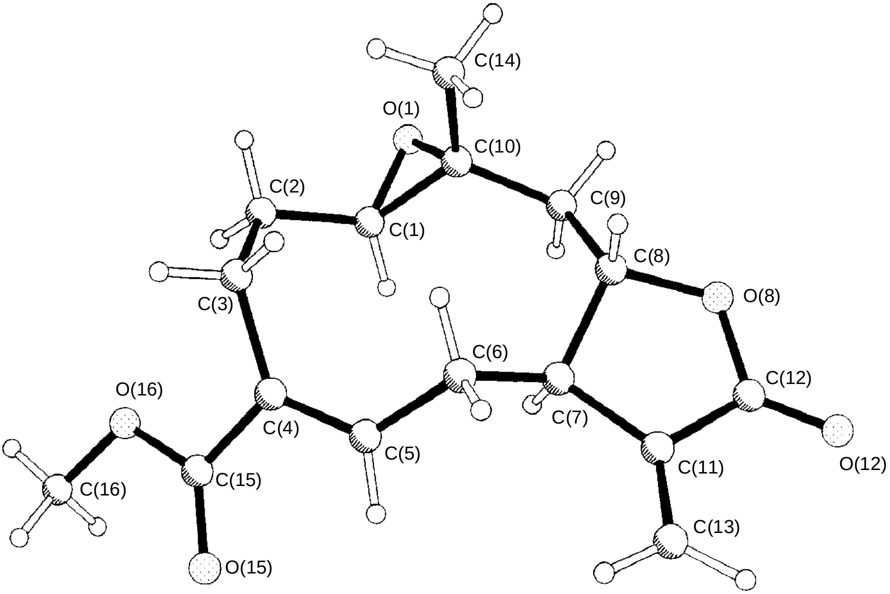

FIG. 1
Perspective view of the β-face of the molecule of hirsutolide (*IV*) and atom numbering scheme

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* The 1(10),4-cyclodecadiene system in germacrane derivatives and its epoxides can be assimilated to a decalin system obtained by formally joining C(10) to C(5). The conformation of such system can thus be described as the conformation of two cyclohexane rings of the decalin system.

ment of the substituents at C(1)–C(10) and C(4)–C(5) can be described by the two-dimensional representation given by Samek and Harmatha[5] as ${}_1D^{14}$, ${}_{15}D_5$. This conformation has been found in several C(6)-lactonized heliangolides: leptocarpin[6], 3β-acetoxy-8β-hydroxy-9β-(2′-methylbutyroxy)heliang-12,6α-olide[7], heliangolidin[8], eupaformonin[9], euparhombin[10], 15-hydroxyacetylleptocarpin[11] and in both crystallographically independent molecules[12] of zaopatanolide B. In Table II the values of the endocyclic torsion angles in hirsutolide have been compared with the values calculated for the global minimum energy form and with the average values obtained for the seven

TABLE I
Atomic coordinates (. 10^4) and equivalent isotropic displacement coefficients (Å^2 . 10^3) of hirsutolide (*IV*)

Atom	*x*	*y*	*z*	U_{eq}[a]
C(1)	−9147(4)	2298(4)	−3466(2)	53(1)
C(2)	−9496(4)	533(5)	−4027(2)	54(1)
C(3)	−9222(3)	−1290(4)	−3469(2)	47(1)
C(4)	−7532(3)	−1435(4)	−2880(2)	44(1)
C(5)	−7042(3)	−913(4)	−2015(2)	46(1)
C(6)	−8438(3)	−88(4)	−1453(2)	48(1)
C(7)	−7859(3)	1918(4)	−1108(2)	46(1)
C(8)	−9454(3)	3405(4)	−1237(2)	49(1)
C(9)	−9764(4)	4417(5)	−2136(2)	62(1)
C(10)	−10521(4)	3181(4)	−2924(2)	55(1)
C(11)	−7219(3)	2141(5)	−118(2)	51(1)
C(12)	−7694(3)	4112(5)	119(2)	55(1)
C(13)	−6477(4)	882(5)	486(2)	67(1)
C(14)	−12488(4)	2426(5)	−2907(2)	67(1)
C(15)	−5669(4)	−2203(4)	−3272(2)	53(1)
C(16)	−4340(4)	−2971(6)	−4591(2)	79(1)
O(1)	−10171(3)	4000(4)	−3781(1)	75(1)
O(8)	−8881(3)	4872(4)	−558(1)	59(1)
O(12)	−7169(3)	5019(4)	785(1)	77(1)
O(15)	−4216(3)	−2714(5)	−2851(1)	101(1)
O(16)	−5907(3)	−2253(4)	−4160(1)	65(1)

[a] Equivalent isotropic displacement coefficient U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

C(6)-lactonized heliangolides mentioned above, representing eight independent measurements. When one compares the ten-membered ring conformation of hirsutolide with another C(8) lactonized heliangolide studied by X-ray methods, i.e. the molecule of scorpioidine (*V*), one notices a remarkable difference. Both crystallographically independent molecules of scorpioidine[13], adopt in the solid state the chair-chair conformation in which the substituents at C(10) and C(4) are *syn* and on the α face of the macrocycle. In Samek and Harmatha[5] notation the conformation of scorpioidine is represented by ${}^{1}D_{14}$, ${}_{15}D_{5}$. Different conformations in C(8)-lactonized heliangolides lead to a different spatial disposition of the crossed double bonds (or their epoxide equivalents) in the cyclodecadiene skeleton. This is illustrated in Fig. 2 from which it is clearly visible that a change from the boat-chair conformation in hirsutolide to the chair-chair conformation in scorpioidine leads to the reversed sterical arrangement of the C(1)–C(10) and C(4)–C(5) bonds. The distance between the two bonds in hirsutolide is 3.436(4) Å, while in scorpioidine such contacts in two independent molecules are 3.177 and 3.163 Å.

Trans double bonds in medium rings are generally subject to significant strain, greater than *cis* double bonds, the effect being still more pronounced for their epoxides. In germacrane sesquiterpenes the average torsion angle moduli around the *trans* double bonds observed in 64 cases is 163.2(5.5)°, while for their epoxides this value, averaged

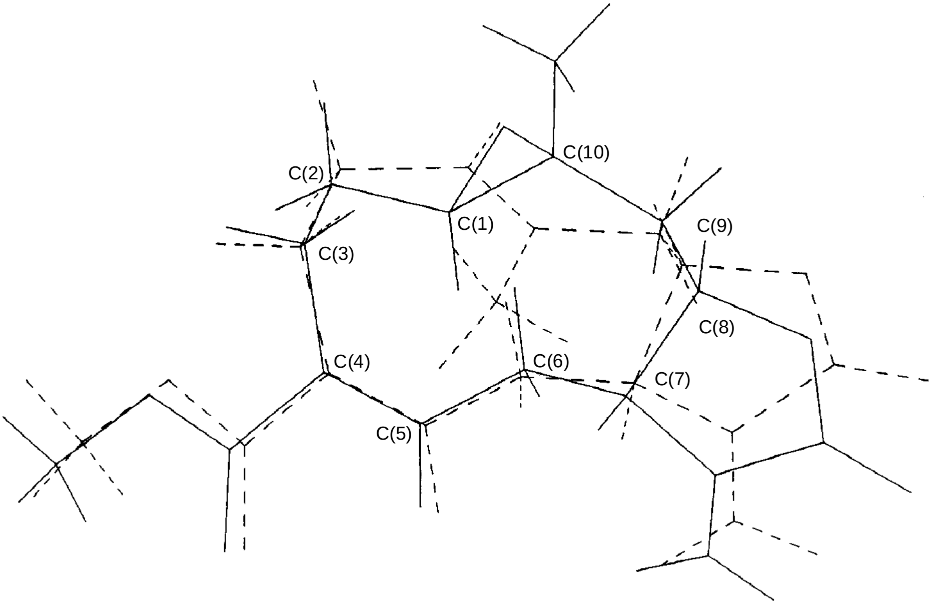


FIG. 2

Pseudoenantiomeric spatial orientation of the C(1)–C(10) and C(4)–C(5) bonds in hirsutolide (*IV*, full line), present work, and in scorpioidine (*V*, dashed line), ref.[13]

over 43 cases, is 153.1(4.4)° retrieved from the Cambridge Structural Database CSD; see ref.[14]. In accord with this the C(9)–C(10)–C(1)–C(2) torsion angle in hirsutolide, 156.1(4)°, is smaller than in scorpioidine (–166.5 and –167.2°). It is interesting to note that in all heliangolides quoted above, both C(6)- and C(8)-lactonized, the sign of the γ-lactone torsion angle at the junction with the macrocycle is always positive. The sign of this torsion angle is in turn paired with the sign of the exocyclic C=C–C=O torsion angle, so that the chirality of the C=C–C=O chromophore and, consequently, the sign of the Cotton effect for the $n \rightarrow \pi^*$ transition[15] should be the same for all aforementioned heliangolides in spite of a different orientation of the γ-lactone (closed at C(6) or C(8)), and a different conformation of the macrocycle (boat-chair or chair-chair). In hirsutolide, the γ-lactone is quite puckered, more than in any of the referenced compounds, the average of the internal torsion angles being 20.0(8.6)°. The conformation can be described as intermediate between C(7), C(8) half-chair and C(7) envelope. The C=C–C=O grouping is decidedly non-planar, the C(13)–C(11)–C(12)–O(12) torsion angle being 17.6(4)°.

Proton and Carbon-13 NMR Spectra

The new NMR measurement of hirsutolide (*IV*) was done at higher frequency (^{1}H at 500 MHz and ^{13}C at 125.7 MHz, respectively) in $CDCl_3$ and C_6D_6 at room and high

TABLE II

Comparison of endocyclic torsion angles (°) in hirsutolide (*IV*) with values calculated for global minimum energy and with the average values obtained for the seven C(6)-lactonized heliangolides

Torsion angle	Present work	Global min. energy	Average value[a]
C(10)–C(1)–C(2)–C(3)	–80.8(3)	–78	–76.0 (12.3)
C(1)–C(2)–C(3)–C(4)	–52.5(3)	–52	–56.8 (6.7)
C(2)–C(3)–C(4)–C(5)	93.8(3)	91	89.6 (5.4)
C(3)–C(4)–C(5)–C(6)	–0.2(4)	–1	2.0 (2.8)
C(4)–C(5)–C(6)–C(7)	–118.7(3)	–116	–121.1 (6.7)
C(5)–C(6)–C(7)–C(8)	132.2(2)	137	135.9 (5.6)
C(6)–C(7)–C(8)–C(9)	–85.4(3)	–85	–78.0 (6.0)
C(7)–C(8)–C(9)–C(10)	68.2(3)	57	57.1 (8.4)
C(8)–C(9)–C(10)–C(1)	–93.9(3)	–98	–93.6 (6.1)
C(9)–C(10)–C(1)–C(2)	156.1(3)	173	167.2 (3.2)

[a] Calculated from literature data mentioned in text.

temperature (50 °C). It allowed us to complete our previously described[2] partial proton NMR data and to correct some erroneously assigned ^{13}C NMR signals.

Proton NMR spectra at room temperature showed line broadening of most of the signals in both solvents. Such behaviour was observed for some other 7,8-lactonized germacranolides with medium rate of interconversion between different conformations of ten-membered carbocyclic ring (for discussion see refs[16,17]). Better resolution was

TABLE III
Proton and carbon-13 NMR parameters (chemical shifts in ppm, coupling constants in Hz) of hirsutolide (*IV*)

Proton NMR				Carbon-13 NMR				
Proton	δ(H)[a]	H$_i$H$_j$[a]	$J(i,j)$[a]	Carbon	δ(C) in CDCl$_3$		δ(C) in C$_6$D$_6$	
					20 °C	50 °C	20 °C	50 °C
H-1	2.83 dd	1,2α	4.3	C-1	60.55	61.15	60.10	60.34
H-2α	1.45 dddd	1,2β	10.5	C-2	26.94	26.98	27.44	27.33
H-2β	2.33 dddt	2α,2β	13.9	C-3	22.66	22.79	22.73	22.75
H-3α	2.61 ddd	2α,3α	6.1	C-4	[b]	131.85	[b]	131.34
H-3β	2.52 bddd	2α,3β	10.4	C-5	[b]	138.86	[b]	139.27
H-5	6.98 dd	2β,3α	4.6	C-6	[b]	30.47	[b]	30.14
H-6α	2.61 b[c]	2β,3β	4.0	C-7	45.87	46.04	45.22	45.39
H-6β	2.67 ddd	2β,9β	0.7	C-8	79.75	79.46	78.78	78.73
H-7	2.69 b[c]	3α,3β	13.6	C-9	45.37	45.55	45.45	45.52
H-8	4.25 ddd	5,6α	7.0	C-10	56.33	56.28	55.69	55.60
H-9α	1.28 bt	5,6β	10.6	C-11	[b]	140.46	[b]	141.35
H-9β	2.80 ddd	6α,6β	14.5	C-12	167.31	167.31	167.08	166.99
H-13	6.32 d	6α,7	[d]	C-13	120.86	120.57	119.03	118.83
H-13′	5.71 d	6β,7	2.1	C-14	17.04	17.09	16.72	16.71
H-14	1.39 s	7,8	8.5	C-15	168.71	168.51	168.02	167.75
COOMe	3.79 s	7,13	3.5	OMe	52.14	52.02	51.53	51.36
		7,13′	3.2					
		8,9α	11.8					
		8,9β	2.7					
		9α,9β	12.9					

[a] In CDCl$_3$ at 50 °C. [b] The signal was not detected. [c] The position of signal was determined from 2D-COSY spectrum. [d] The *J*-value could not be determined (H-6α and H-7 give very broad unresolved signals).

achieved at 50 °C namely in $CDCl_3$ solution where all signals (except of H-6α and H-7 protons) give well resolved multiplets. The presence of protons H-6α and H-7 as broad humps was manifested on integration curve in 1D NMR spectrum and their chemical shift values were determined from corresponding cross-peak positions in 2D-COSY spectrum. Proton NMR parameters of hirsutolide (*IV*) in $CDCl_3$ at 50 °C are summarized in Table III. To obtain some more information on conformation behaviour we have measured a low temperature ^1H NMR spectra in CD_2Cl_2 at 0, –20, –40 and –60 °C. The spectra at the lower temperatures (–40 and –60 °C) showed two sets of signals for the most of protons in the intensity ratio ca 3 : 1. Extremely crowded region of spectrum between 2.2 and 2.9 ppm (containing eight protons of the molecule) did not allowed to assign signals of some protons belonging to a minor conformer. The situation is illustrated in Fig. 3 showing ^1H NMR spectra of hirsutolide (*IV*) obtained at +20 and –60 °C.

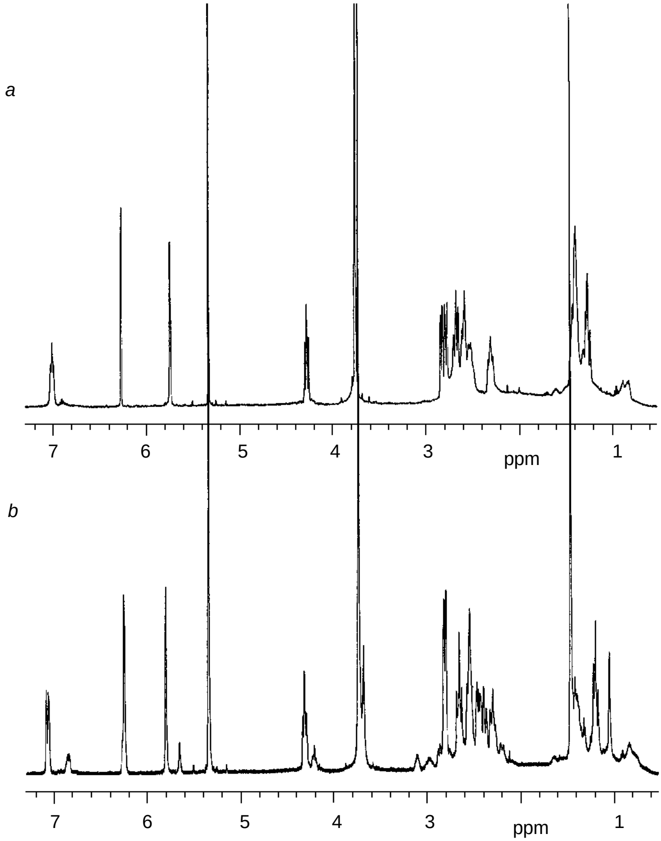


FIG. 3
^1H NMR spectrum of hirsutolide (*IV*) in CD_2Cl_2: *a* at +20 °C, *b* at –60 °C

The signal positions in time-averaged spectrum (at +20 °C) well fit the ones calculated as the sum of population weighted chemical shifts of two conformers observed at –60 °C. Proton NMR parameters derived are given in Table IV together with interproton torsion angles calculated from the X-ray data of hirsutolide (*IV*) and scorpioidine (*V*) in crystal. A good agreement between the observed vicinal coupling constants and the interproton torsion angles for *IV* indicates that major conformer corresponds to the one observed in

TABLE IV
Proton NMR parameters (chemical shifts in ppm, coupling constants in Hz) of hirsutolide (*IV*) in CD_2Cl_2 and interproton torsion angles calculated from X-ray data of hirsutolide (*IV*) and scorpioidine (*V*)

Proton	Chemical shifts			H_iH_j	Coupling constants			Torsion angles	
	at +20 °C	at –60 °C			at +20 °C	at –60 °C		*IV*	*V*
		major	minor			major	minor		
H-1	2.83	~2.81	2.87	1,2α	4.4	3.9	[a]	–50	166
H-2α	1.43	1.39	~1.60	1,2β	10.5	12	[a]	–167	48
H-2β	2.31	2.30	[a]	2α,2β	13.9	14	[a]	–	–
H-3α	2.60	2.55	~2.76	2α,3α	5.3	5	[a]	–55	52
H-3β	2.53	2.39	2.97	2α,3β	10.2	12.4	[a]	–172	174
H-5	7.01	7.07	6.85	2β,3α	4.9	5	[a]	62	–65
H-6α	[a]	2.44	[a]	2β,3β	4	4	[a]	–54	52
H-6β	2.67	2.65	[a]	2β,9β	<1	<1	[a]	–	–
H-7	[a]	2.53	[a]	3α,3β	13.7	14	[a]	–	–
H-8	4.28	4.31	4.21	5,6α	[a]	5	5	–60	–68
H-9α	1.26	1.19	[a]	5,6β	10.5	13.2	~11.5	–178	–178
H-9β	2.78	2.81	[a]	6α,6β	14.5	14.2	[a]	–	–
H-13	6.27	6.24	6.27	6α,7	[a]	7.6	[a]	133	104
H-13′	5.75	5.80	5.65	6β,7	[a]	<2	[a]	–109	–144
H-14	1.39	1.46	1.04	7,8	8.6	8.7	8.4	160	154
COOMe	3.76	3.73	3.68	7,13	3.6	3.7	3.5	–	–
				7,13′	3.2	3.2	3	–	–
				8,9α	11.7	11.8	~11.0	–178	168
				8,9β	2.5	3.3	3	65	50
				9α,9β	12.7	12.7	[a]	–	–

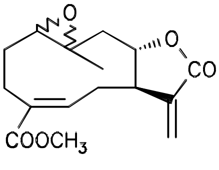
[a] The value of parameter was not determined.

crystal. A very poor set of *J*-values for a minor conformer does not allow its sufficient geometry characterization. Nevertheless at least some the observed differences between time-averaged *J*-values at 20 °C and the ones of major conformer at –60 °C can result from the contribution of *J*-values of minor conformer if it adopts a geometry corresponding to one found for scorpioidine (*V*) in crystal.

Similar line-broadening effects have been observed also in ^{13}C NMR spectra of hirsutolide (*IV*) in $CDCl_3$ and C_6D_6. At room temperature the signals of carbons C-4, C-5, C-6 and C-11 could not be detected and very weak signals were observed for carbons C-1 and C-8. High temperature spectra (at 50 °C) allowed to identify the signals of all 16 carbons present although some of them (C-6 and C-4) are still broadened and weak. In this connection we should notice that for these two carbons the uncorrect chemical shift values in C_6D_6 has appeared in our previous paper[2]. Carbon-13 chemical shifts in both solvents are summarized in Table III.

The structure of hirsutolide shown in formula *IV* represents also its absolute configuration as it follows from X-ray determined relative configuration and CD spectrum[2] ($n\rightarrow\pi^*$ transition of α-exomethylene-γ-lactone chromophor).

Some years ago Bohlmann et al.[18] had isolated from *Arctotis arctoides* a compound for which they suggested (mainly on the basis of ^{1}H NMR data) the identical structure *IV*. The detailed comparison of ^{1}H and ^{13}C NMR data strongly suggest the identity of both compounds. Our ^{13}C NMR spectra obtained by APT method[19] showed the neccessity to interchange the assignment of three pairs of carbon signals – C-1/C-6, C-5/C-11 and C-7/C-9 – in literature data[18] on compound *IV*.



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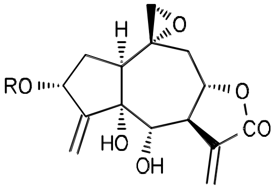
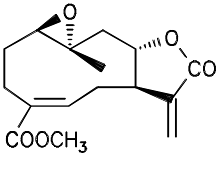
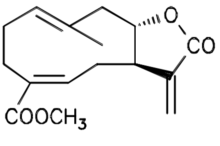


II, R = $COCH_2CH(CH_3)_2$
III, $COCH(CH_3)CH_2CH_3$



IV



V

EXPERIMENTAL

Crystal data: $C_{16}H_{20}O_5$, m.p. 110 – 112 °C, monoclinic, space group $P2_1$, $a = 7.095(1)$, $b = 7.026(1)$, $c = 14.949(3)$ Å, $\beta = 96.84(3)°$, $V = 739.9(4)$ Å^3, $D_x = 1.312$ g cm^{-3}, $Z = 2$, $\lambda(\mathrm{CuK}\alpha) = 1.54178$ Å, $\mu = 0.80$ mm^{-1}.

Crystallographic measurements: A crystal of approximate dimensions 0.20 × 0.30 × 0.45 mm grown from propanol was used for data collection on a KM-4 diffractometer using graphite monochromated CuKα radiation. Lattice constants and an orientation matrix were obtained from a least-squares fit of 34 centered reflections. Intensities were measured using $\theta - 2\theta$ scan technique for 2θ from 2 to 130°, with the scan rate depending directly on the net count obtained on rapid pre-scans for each reflection and a scan range in ω of 1.35° plus K_α separation. Background measurements were estimated from 96-step profile. Two standard reflections were monitored after collection of every 100 reflections and their intensity variation was not greater than 3%. A half of the reciprocal sphere was collected in the range h: $-8 \rightarrow 8$, k: $-8 \rightarrow 8$, l: $0 \rightarrow 17$. 2 368 independent reflections were measured (Bijvoet pairs not averaged) of which 1 988 were considered observed [$F > 4\sigma(F)$]. Lorentz and polarization factors were applied in reducing the intensities to structure factor amplitudes[20], but no absorption correction was deemed necessary [$\mu(\mathrm{CuK}\alpha) = 0.80$ mm^{-1}].

Structure analysis: The structure was solved by direct methods using SHELXS86 (ref.[21]) and refined using F magnitudes by full-matrix least squares of SHELX76 (ref.[22]). Non-hydrogen atoms were refined anisotropically. The H-atoms were placed at calculated positions and were subjected to constrained refinement with a common isotropic displacement parameter $U = 0.075$ Å^2. In the final stages of the refinement an empirical isotropic extinction parameter x was introduced to correct the calculated structure factors by multiplying them by a factor $1 - xF_c^2/\sin\theta$ and it refined to a value $1.5(5) \cdot 10^{-6}$. The function minimized was $\Sigma w((|F_o| - |F_c|)^2)$ with $w = 1/[\sigma^2(F_o) + 0.0003F_o^2]$ where $\sigma(F_o)$ is the standard deviation of the observed amplitudes, based on counting statistics. A convergence was attained at $R = 0.035$ ($wR = 0.044$) for 1 988 observed reflections and 190 refined parameters. The final difference map showed minima and maxima ranging from -0.26 to 0.28 e Å^{-3}.

NMR spectra: The proton and carbon-13 NMR spectra of hirsutolide (*IV*) were measured on a Varian UNITY 500 spectrometer (^{1}H at 500 and ^{13}C at 125.7 MHz frequency) in $CDCl_3$ and C_6D_6 solution at temperature 20 and 50 °C. Low temperature ^{1}H NMR measurement was done in CD_2Cl_2 at 20, –20, –40 and –60 °C. Proton NMR spectra were referenced to either internal TMS (in $CDCl_3$) or residual solvent signal (in C_6D_6, $\delta(C_6D_5H) = 7.27$ ppm and in CD_2Cl_2, $\delta(CHDCl_2) = 5.33$ ppm); carbon-13 spectra to solvent peaks ($\delta(CDCl_3) = 77.0$ ppm and $\delta(C_6D_6) = 128.0$ ppm, respectively). 2D-COSY technique[23] was used for the structural assignment of proton signals. Carbon-13 NMR signals were classified according to the number of directly bonded protons using APT pulse sequence[19].

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